(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 March 2004 (11.03.2004)

PCT

(10) International Publication Number
WO 2004/020604 A3

(51) International Patent Classification[7]: C12P 19/34

(21) International Application Number:
PCT/US2003/027286

(22) International Filing Date: 29 August 2003 (29.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/406,894 29 August 2002 (29.08.2002) US

(71) Applicant: AMERSHAM BIOSCIENCES CORP [US/US]; 800 Centennial Avenue, Piscataway, NJ 08855 (US).

(72) Inventors: SOOD, Anup; 37 Honeyman Drive, Flemington, NJ 08822 (US). KUMAR, Shiv; 21 Muirhead Court, Belle Mead, NJ 08502 (US). FULLER, Carl; 33 Robbins Avenue, Berkeley Heights, NJ 07922 (US). NELSON, John; 16 Marshall Road, Hillsborough, NJ 08844 (US). MACKLIN, John; 104 North Monroe Avenue, Wenonah, NJ 08090 (US).

(74) Agents: JI, Yonggang et al.; Amersham Biosciences Corp, 800 Centennial Avenue, Piscataway, NJ 08855 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
12 August 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ALLELE SPECIFIC PRIMER EXTENSION

(57) Abstract: A method of characterizing a nucleic acid sample is provided that includes the steps of: (a) conducting a DNA polymerase reaction that includes the reaction of a template, an allele specific primer, at least one terminal phosphate-labeled nucleotide, DNA polymerase, and optionally an enzyme having 3' ? 5' exonuclease activity when the primer is non-hydrolyzable, which reaction results in the production of labeled polyphosphate; (b) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species; (c) detecting the detectable species; and (d) characterizing the nucleic acid sample based on such detection.

WO 2004/020604 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/27286

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12P 19/34
US CL : 435/91.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.1, 91.2, 183; 436/94; 536/23.1, 24.3, 24.33, 25.3, 25.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,255,083 B1 (WILLIAMS) 03 July 2001 (03.07.2001), see entire document, especially see columns 2, 3, and 19-21.	1, 8-10, and 13 ---------- 5-7, 11, 14, 15, and 24-28
Y	US 5,518,900 A (NIKIFOROV et al) 21 May 1996 (21.05.1996), see entire document, especially see column 4, lines 56-67 and column 13, lines 51-65.	5-7, 14, and 15
Y	US 5,759,772 A (KIRKPATRICK et al) 02 June 1998 (02.06.1998), see column 3, lines 24-36.	11
Y	US 5,270,185 A (MARGOLSKEE) 14 December 1993 (14.12.1993), see column 7.	24-27
A,P	US 2003/0134807 A1 (HARDIN et al.) 17 July 2003 (17.07.2003), see entire document,especially see [0223].	1-4, 8-10, 12, 13, and 28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
15 June 2004 (15.06.2004)

Date of mailing of the international search report
02 JUL 2004

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Frank Lu

Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
STN and EAST
Search terms: polymerase, pyrophosphate or ppi, primer, phosphatase, terminal or end, and sequencing